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Survey of viruses infecting garlic in Samsun, Turkey

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Abstract

Garlic is one of the most important *Allium* crops in Turkey. A survey for viruses infecting garlic (*Allium sativum* L.) was carried out in Samsun, Turkey. A total of 70 samples were collected and checked by biological and serological methods for the presence of *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV), *Garlic common latent virus* (GarCLV), *Shallot latent virus* (SLV), and *Iris yellow spot virus* (IYSV) using virus-specific antiserum. OYDV was detected by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) and bioassay in garlic (5.7%) samples collected from Samsun, Turkey.

Keywords: allium, garlic, *Onion yellow dwarf virus*, survey, viruses

Introduction

The *Allium* genus includes onion, garlic, leek, chives, shallots, and scallions, and was previously classified in the Alliaceae family (Friesen et al., 2006), now classified in the Allioideae subfamily within the Amaryllidaceae family (Chase et al., 2009).

Garlic (*A. sativum* L.) and other edible *Allium* are among the oldest cultivated plants, and are used for multiple purposes (Borde et al., 2009). Of the over 50 diseases that affect cultivated *Allium* crops (Dugan, 2007; Schwartz and Mohan, 2008), there are four viruses consistently documented as infecting garlic; *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV), *Garlic common latent virus* (GarCLV), and *Shallot latent virus* (SLV) (Dovas et al., 2001; Fajardo et al., 2001).

Some viruses, especially, OYDV and LYSV, cause mosaic symptoms in infected leaves and can cause yield reductions in excess of 25% (Walkey and Antil, 1989). These are the most important viruses in terms of the damage, with garlic bulb weight reduced between 24 and 60% for OYDV and between 17 and 54% for LYSV (Lot et al., 1998). OYDV has been considered a main viral pathogen, on the basis of its belonging to the *Potyvirus* genus, which is known to include many highly pathogenic viruses (Shukla et al., 1994). Aphids play an important role as vector to spread potyviruses (Garzo et al., 2004).

Garlic and onion are the most important *Allium* crops in Turkey. The Black Sea Region has a big share of garlic production in Turkey. There has been no comprehensive study for viral diseases of garlic crops in Samsun province, located in the Black Sea region. The present study was carried out to determine which viruses affect garlic crops in the major production areas of the middle Black Sea Region.

Materials and methods

Commercial garlic fields were selected randomly from various locations in Samsun province. Field inspections were conducted during two consecutive vegetation periods in Bafra, Carsamba, and İlkadim districts. Disease occurrences were visually assessed in the field and the identities of the pathogens were confirmed by laboratory testing using biological and serological tests.

Garlic leaf samples were collected randomly along field diagonals, and 2-3 leaves from each plant were taken. A total of 70 samples were obtained from all sampling sites. Within 24 h after harvest, leaves were kept at –70 ºC until the serological tests were performed.

All samples were tested using double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) kits (Bioreba and Agdia) for the presence of the following viruses; *Onion yellow dwarf virus*, *Leek yellow stripe virus*, *Garlic common latent virus*, *Shallot latent virus*, and *Iris yellow spot virus*. The procedure was done according to the manufacturer's instructions (Bioreba and Agdia) for all viruses. ELISA was performed in 96-well polystyrene plates (Nunc MaxiSorp, Denmark) and two replications were used to load samples on the plates. Leaf extracts were prepared by grinding 1 g of leaves in 5 ml of extraction buffer (pH 7.4) containing 2% polyvinylpyrrolidone (PVP), 0.1% skimmed milk powder, and 0.05% Tween-20. Plates were developed 100 µl of 1 mg/ml *p*-nitrophenyl phosphate (Sigma-Aldrich) dissolved in 0.01 M diethanolamine buffer (pH 9.6) and read using a spectrophotometer (Tecan Spectra II, Grodig/Salzburg, Austria), with readings three times that of the negative controls considered positive for the virus (Mullis et al., 2004).

Mechanical inoculations were carried out by rubbing sap from virus source plants that gave high absorbance values in ELISA on leaves of test plants dusted with carborundum. Sap was prepared by grinding infected plants with a mortar and pestle with 0.05 M phosphate buffer (pH 7) (Conci et al., 1992). The isolates were mechanically inoculated onto five plants of each *Nicotiana tabacum* "Samsun", *Chenopodium quinoa*, and *A. cepa*, maintained in a plant growth room at 23-25 °C and monitored daily for symptoms. Inoculated plants were tested for the presence of virus by DAS-ELISA to confirm the results of previous serological testing.

Results and discussion

A survey of virus diseases was carried out in garlic-growing areas in the middle of the Black Sea Region of Turkey. Leaf samples with symptoms (mosaic consisting of light and dark green stripes along the leaves) suggesting the virus presence and symptomless samples were collected randomly.

Altogether, 70 leaf samples were examined by DAS-ELISA. Based on serological tests, only OYDV was detected in garlic samples. The other viruses like LYSV, GarCLV, SLV and IYSV were not detected in any of the samples tested in the current study (Table 1).

Table 1. Occurrence of OYDV in garlic samples

Regions	No. of samples tested	OYDV (%)[a]	LYSV, GarCLV, SLV, IYSV
Bafra	40	7.5	-
Carsamba	20	5	-
İlkadim	10	0	-

[a] OYDV: *Onion yellow dwarf virus,* LYSV: *Leek yellow stripe virus*, GarCLV: *Garlic common latent virus*, SLV: *Shallot latent virus*, IYSV: *Iris yellow spot virus*

OYDV was identified in 5.7% of garlic samples. The highest infection rate was found in garlic crops in Bafra (7.5%), followed by garlic-producing areas in Carsamba (5%). OYDV was not detected in tested garlic samples from Samsun-İlkadim.

In garlic plants, single or mixed infections in combination with LYSV, GarCLV, SLV, and IYSV were not detected by serological methods in the current study. Positive findings for OYDV were confirmed in biological tests to confirm the findings in ELISA. In the present study, OYDV was isolated from naturally infected garlic plants and the isolate was mechanically transmitted to test plants. Typical symptoms of the virus, such as yellow lines, were observed on leaves of *A. cepa* 21 days after mechanical inoculation (dpi) with the OYDV. *C. quinoa* plants reacted with local lesions of inoculated leaves 10 dpi, but the isolate could not be mechanically transmitted to *N. tabacum "*Samsun*"* in current study.

OYDV, the *Potyvirus* genus, seems to be spread in the garlic growing areas in Samsun province and in many other countries around the world causing significant degeneration of the crop after only a few multiplications under field conditions (Fajardo et al., 2001; Takaichi et al., 2001). The results show that OYDV is common on garlic crop in Samsun province. LYSV was not detected in the samples analyzed. Similar results were obtained by Klukackova et al. (2007). The results showed that Czech seed garlic (means from five cultivars) was infected on average at 75.4% by OYDV. LYSV was detected at low frequencies in 3 of 5 Czech cultivars. Partially similar results were obtained by Dovas et al. (2001), who proved incidence of viruses infecting garlic, leek and onion crops in Greece. The high incidence of OYDV (98%), and LYSV (84%) was detected by Dovas et al. (2001) contrast to the current study results.

The *Potyvirus* genus is the largest and economically most important group of plant viruses (Ward and Shukla, 1991). OYDV, an aphid-borne *Potyvirus*, is one of the

major viral pathogens of onion and garlic (Mahmoud et al., 2008). Details of the occurrence of OYDV and LYSV have been described in Greece (Dovas et al., 2001), Brazil (Fajardo et al., 2001), Japan (Takaichi et al., 2001), and Italy (Dovas and Vovlas, 2003), but LYSV was not detected in tested onion and garlic samples in the current study.

The rates of garlic infection prevalence are related the size of aphid populations in a particular region (Takaichi et al., 1998). In Turkey, garlic is generally grown in the cold and wet conditions during the fall and winter seasons when the population of aphid is low. In the present study, OYDV was detected by ELISA in symptomatic leaves only in the Black Sea Region of Turkey and showed low incidence, most probably due to the reduced vector populations and activity.

Conclusions

In conclusion, this is the first report of a survey using serological and biological diagnostic procedures to identify the viruses of garlic crops in Samsun, Turkey. The results obtained in the investigation demonstrate that the low percentage of garlic plants was found infected with OYDV in the Middle Black Sea Region of Turkey. The information presented in the current study will hopefully aid in improving control strategies for the virus infections.

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